Human sperm survival bioassay to examine toxicity of a new clinical laboratory equipment disinfectant

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Objective

To test potential sperm toxicity of a newly available incubator and laminar flow hood disinfectant

Introduction

Human sperm survival assay is one of the most commonly used bioassay methods for assisted reproductive technology (ART) laboratory quality control and proficiency testing (1, 2). This bioassay is convenient for clinical andrology and embryology laboratories to perform with materials readily available and protocols easy to implement. Mouse embryo assay (MEA) (3), another bioassay commonly used in ART laboratories, has only modest sensitivity and consistency (4-6). Sperm survival assay is readily accessible for many ART laboratories, and can serve as a complementary method to MEA for toxicity assessment (2, 7, 8).

A newly developed incubator and laminar flow hood disinfectant which contains didecyldimethylammonium chloride, benzyl-C12-18-alkyl-dimethyl-chloride and C12-14-alkyl(methyl-dimethyl)-chloride, was recently made available under the commercial name Oosafe® (SparMED ApS, Farum, Denmark).

In this study, we tested the potential toxicity by using a human sperm survival assay to examine the feasibility of applying such disinfectant in our andrology and embryology laboratories, as well as establish useful reference data for other laboratories which may apply this disinfectant.

Materials & Methods

Human Sperm Survival Assay

Human sperm specimens were cultured in duplicates in Nunc 4-well IVF plates, in P1 medium (Irvine Scientific, Santa Ana, CA) supplemented with 5% Synthetic Serum Substitute (Irvine Scientific) without oil overlay. Disinfectant treated groups included Treatment Group 1 (T1, n=18) at 37°C with humidified triple-gas (5% CO_2, 5% O_2 and 90% N_2), and Treatment Group 2 (T2, n=17) at room temperature with humidified triple-gas. Control groups C1 (n=18) and C2 (n=17) were cultured with triple gas at 37°C and RT, respectively, without disinfectant treatment.

Materials & Methods (cont.)

A third treatment group (T3) was cultured in 70% ethanol-treated plates at RT with triple gas, to obtain a baseline sperm survival in a defined toxic environment. Sperm motility (%), major progression grades (0-4) and sperm motility index (SMI) values were examined at 24 hr intervals up to 72 hrs. A SMI < 0.75 was considered toxic to the sperm.

Statistical Analysis

Analysis of variance (ANOVA) for repeated measures was used to evaluate the statistical significance of changes in the paired sperm measures using a between-within design. Measurements were repeated every 24 hours up until 72 hours after an initial reference observation (yielding Initial, 24-hour, 48-hour, and 72-hour measures). ANOVA produced between-group comparisons of (1) the groups at each time point, (2) their change from the mean of prior time point measurements, and (3) the slopes of values for each group. All statistical significance was set at two-tailed 95% ($P < 0.05$) using Bonferonni's adjustment for multiple comparisons.

Results

Sperm motility, major progression, and sperm motility index (SMI) values were evaluated. Overall, sperm motility decreased through the testing period, with the largest change occurred between 24-48 hr. There were no significant differences between treatment and control groups at both 37°C and RT (**Figure 1** and **Table 1**). When comparing treatment and control groups cultured at 37°C, there was no significant difference in sperm motility at 24 hr, 48 hr and 72 hr and motility declines during 0-24hr, 24-48hr, and 24-72 hr. At RT, we found no significant difference in sperm motility at each time point or motility declines between time points in the treatment and control groups. Temperature significantly affected the slope of sperm survival through the testing period, in all conditions regardless disinfectant treatments. SMI remained in the acceptable range in treatment and control groups at both temperature conditions. Ethanol treatment resulted in severe toxicity compared to all other groups. Sperm cultured in ethanol treated plates showed dramatic decrease of motility and progression compared to all 4 treatment and control groups.

Results (cont.)

Table 1: Sperm motility (mean±S.D.) values after exposure to media of treatment and control groups

Motility (%)	**Initial**	**24 hr**	**48 hr**	**72 hr**
Treatment 1 (T1)	84.7±9.3	68.9±15.5	33.2±18.6	3.9±3.7
Control 1 (C1)	84.7±9.3	70.1±13.7	35.7±17.7	4.2±4.2
Treatment 2 (T2)	85.6±9.8	75.9±12.5	65.7±15.4	59.3±13.7
Control 2 (C2)	85.6±9.8	75.7±12.7	66.3±15.1	60.5±14.3
Treatment 3 (T3)	83.8±12.8	3.4±4.0	0±0	N/A
Progression	**Initial**	**24 hr**	**48 hr**	**72 hr**
Treatment 1 (T1)	3.61±0.50	3.56±0.54	2.92±0.90	0.97±0.99
Control 1 (C1)	3.61±0.50	3.64±0.48	2.69±0.93	1.16±1.19
Treatment 2 (T2)	3.59±0.51	3.00±0.61	2.91±0.67	2.87±0.72
Control 2 (C2)	3.59±0.51	2.97±0.62	2.91±0.64	2.80±0.77
Treatment 3 (T3)	3.75±0.46	0.56±0.73	0±0	N/A
SMI		**24 hr**	**48 hr**	**72 hr**
T1/C1		0.98±0.095	0.94±0.172	1.01±0.338
T2/C2		1.00±0.033	0.99±0.060	0.98±0.077
T3/C2		0.05±0.050	0±0	N/A

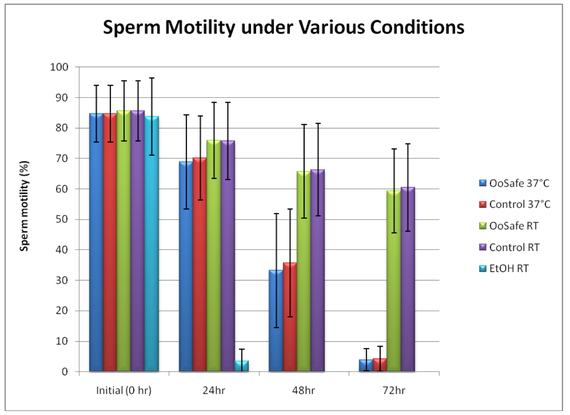

Figure 1. Sperm motility under various culture conditions at different time points

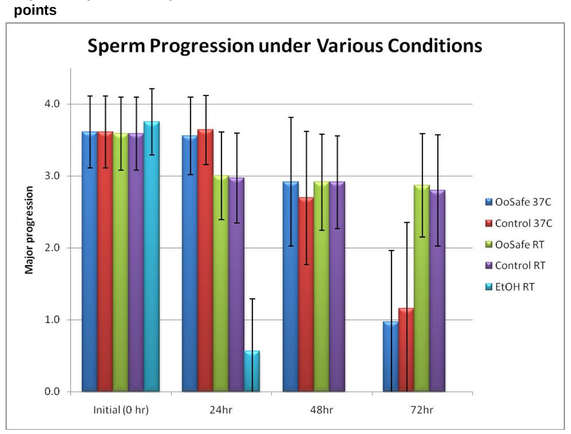

Figure 2. Sperm progression under various culture conditions at different time points

Results (cont.)

Sperm progression decreased through the testing period in all groups. The slopes obtained from all groups at both 37°C and RT showed no significant difference between the treatment and control groups (**Figure 2** and **Table 1**). We found no significant difference in sperm progression between the treatment and control groups at 37°C at 24 hr, 48 hr and 72 hr, as well as the slope between time points through the culture period. No significance was found in sperm progression between the treatment and control groups cultured at RT at each time point as well as between time points. Temperature significantly affected the slope of sperm progression through the testing period, in all conditions regardless disinfectant treatments, similar to the findings from sperm motility results described above.

Conclusion

In this study we compared sperm survival in various conditions. Temperature remained the major factor affecting sperm survival and major progression over time. Treatment with the disinfectant did not significantly decrease sperm survival and major progression as compared to control groups at both 37°C and room temperature. We conclude that the chemical disinfectant Oosafe® is not detrimental to sperm motility and progression.

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